

## *Just the Facts...* **Rodent Spermatozoa Analysis (RSA) in Health Risk Assessment**

Rodent Spermatozoa Analysis, better known as Rodent Sperm Analysis (RSA) is a relatively new field-applied health risk assessment method developed within the CHPPM. Adapted from an established laboratory procedure, it provides immediate benefits to the Army in evaluating the health status of animals exposed to contaminated soil at installations, and it holds great potential to serve as a tool that screens for reproductive effects in humans (civilians and Soldiers) who may be exposed to soils contaminated from Army operations.

### **How Does RSA Work?**

RSA relies on the body of scientific literature that exists for the sperm quality of common laboratory species (mice, rats); as it relates to the ability to successfully reproduce. Sperm count, sperm motility (ability of sperm to swim normally), and sperm morphology (the percentage of misshapen sperm) are known barometers of reproductive success. That is, we know how much of a reduction in the count and motility, and how much of an increase in the number of abnormally-shaped sperm, will trigger reduced reproductive success. Because of the potential for drugs that are under development to produce reproductive side effects (e.g., sterility) in the people who would take them, the three sperm parameters are monitored daily in scores of animal studies conducted at pharmaceutical laboratories. In RSA, this standard sperm monitoring procedure is advanced to the field, to assess the potential of contaminated soils to function as reproductive toxicants in ecological receptors.

### **RSA's Role in Ecological Risk Assessments**

In most cases, ecological risk assessments only extend to the desktop calculation stage. Although guidance recommends that the field condition be assessed in order to verify the commonplace predictions of moderate to severe impacts accruing to the site biota, rarely do field studies proceed. Furthermore, when field efforts *do* occur, these are generally not directed at addressing the issues raised by the desktop exercise. In developing RSA, USACHPPM realized the many advantages there are to working with small rodents from the field. Mice, rats, voles, squirrels, and other similar mammalian species, all suited to RSA, are found in nearly every habitat, and all are relatively easy to trap and to handle. Securing permission from animal care and use committees to collect and sacrifice the 10 to 15 animals needed from a site of interest, is ordinarily not a difficulty. Most importantly, small rodents represent a potential worst case for chemical exposure at a contaminated site; because they are non-migratory, spend all of their time on the ground or beneath it, and consume all of their diet from the contaminated site. If these highly exposed small rodents are not demonstrating reproductive impacts, it can be reasoned that other terrestrial receptors that have much less direct contact with the ground and that forage over distances that far exceed the contaminated land parcels, are also not experiencing compromised reproductive success. During the course of an RSA's brief (two-week) field effort, an abundance of population data (e.g., species diversity, sex ratio, age distribution, etc.) and tissue analysis data is also collected. This information is used to corroborate the findings of the sperm parameter analysis conducted for a contaminated site relative to a matched reference (non-contaminated) location.

### **RSA's Potential Role in Human Health Risk Assessment and Soldier Protection**

The vast majority of our understanding of chemical toxicity in humans derives from testing conducted with laboratory animals, principally the laboratory rat. In developing RSA, USACHPPM reasoned that if for human health protection, we ordinarily rely on the results of chemical exposures to rodents that are administered under artificial conditions in the laboratory; we should certainly be able to extrapolate from the field rodent's natural condition to an actual or intended human exposure at a contaminated site. Commonly, laboratory testing with rodents involves single-generation studies only. The advantage to assessing the reproductive health condition of wild rodents is that they may easily reflect 100 generations of exposure, owing to many Army properties having decades-old contamination. Finding that reproductive health impacts are absent in such chronically exposed animals, could well provide confidence that a similarly exposed human would not develop a reproductive effect. That the Army is concerned with the full spectrum of health effects to military personnel, to include reproductive effects, is evident in a number of ways. The Army has collaborated with the National Institute for Occupational Safety and Health to address the concern, and numerous studies have already been conducted, including one evaluating Vietnam War veterans who had been exposed to Agent Orange (Operation Ranch Hand) and several other studies directed at the exposures of individuals to solvents and fuels during aircraft maintenance duties.

### **RSA Application**

RSA can be applied at almost any soil-contaminated site with health risk assessment concerns, because of the ubiquitous distribution of small rodents. The method has utility at sites managed under a multiplicity of Army programs (Environmental Restoration, BRAC, FUDS, etc.). To date, RSA has been used at nine installations including two Superfund NPL sites, to assess the health status of the ecological community. The majority of Army applications have been at active artillery ranges for one component of the Army's Sustainable Ranges Program. In each of these applications, the sperm parameter testing has proven to be a sufficiently sensitive measure. Importantly in each instance, the

RSA results have indicated that despite years of artillery detonation, ecological health effects are not present. This may suggest that rodents, and by extension other receptors, are not being impacted by the site contamination. RSA is able to make such a claim because reproduction is reputedly the highest valued toxicological endpoint in ecological risk assessment.

### **Benefits to the Army from RSA Application**

For Army risk assessments, RSA offers several advantages over the conventional approaches to study. For ecological assessments, RSA supplies the needed exit strategy for sites that would otherwise stand to have an ongoing series of tests run. The method draws as definitive a determination about the health status of receptors as is possible, although it costs a fraction of what a conventional risk assessment would. RSA can therefore save the Army millions of dollars in obviating the need for additional unnecessary testing, and also in discovering that soil cleanups are not needed. Regarding human health assessments, USACHPPM will be investigating how RSA can support the Soldier readiness mission, hopefully providing an early indication of the potential for harmful effects to accrue to the Soldier assigned to his or her particular tour of duty. A very noteworthy benefit of RSA is its rapid turn-around of information. Within one month of completing a two-week field effort, the RSA results are known.

